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<p>The diffusion of proton in thin water layers was monitored by time resolved fluorescence of pyranine. The observed dynamics was analyzed by numerical integration of the Debye Smoluchowski differential equation for a proton diffusing in an electric force field enclosed between two planar surfaces kept at constant distance apart and having a finite affinity for the proton.</p> <p>The results clearly imply that as the width of the aqueous layer is less than $\sim 12\text{\AA}$, the proton is effectively confined to the immediate environment of origin. There is a direct correlation between the measured diffusion coefficient and the activity of water in the aqueous layer. This observation may reveal the nature of ordering of water molecules by the hydration forces. <i>Keywords:</i></p>					
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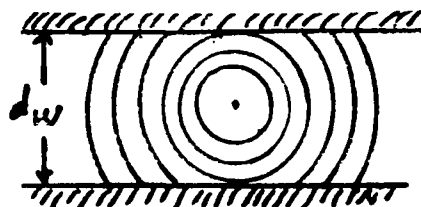
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The massive ion flux driven by membranal enzymes is discharged into microscopic cavities. In the case of mitochondria, chloroplast or retina cells, the receiving lumen has a shape of thin body of water, 1.5-5 nm deep and surface dimension measured in micrometer units. Our studies, during the past year, were aimed to quantitate how this shape of the reaction space affects the dynamics of proton transfer.

Three parameters were investigated:

- 1) The effect of the width of the water layer on the dynamics of proton diffusion;
- 2) The effect of binding to surface on the mechanism propagation of proton near the surface;
- 3) The effect of order parameter of the water, in the immediate vicinity of the membrane, on the diffusion coefficient of proton.



Scheme 1

These problems were investigated experimentally using the subnanosecond dynamic measurements of the geminate recombination between proton and the excited anion (πO^{*-}) of pyranine (8 hydroxy pyrene 1,3,6 trisulfonate). The adequacy of reaction for the pertinent system is detailed below.

1. The effect of geometric restriction: As seen in Scheme 1, when proton, released at a given point, diffuses a distance $r < (dw/2)$ its diffusion space is spheric symmetric. At $r > dw/2$ the diffusion space is annular with smaller volume increment per step. Thus dw becomes a parameter affecting the dynamics of the reaction.

2) The effect of a reactive surface: At a distance $r > dw/2$ the proton has a finite probability to encounter a phospho anion of the phosphatidylcholine lipids used in our experiments. The probability of this reaction is function of the phospho moieties density on surface, the contact area of the annular ring and an intrinsic property of the reactant. Any proton reacting with the surface group lowers the probability density of protons around the anion and thus affect the experimental observation.

3) The effect of ordered water: The hydration forces orient the water molecules at the interface. The diffusion of proton in water is affected by the ordering of the water molecules. The diffusion coefficient is given by $D = 6 d^2 \nu$ where ν is the frequency at which a proton executes a random step with a length d . Proton diffusion by the so-called "Grottuss mechanism" is carried in quantized steps of one or more water molecule diameters. Thus ordering of water molecule will provide a larger step unit (if ordering is mostly correlation of water molecules

along a spatial axis). If, on the other hand, ordering is favoring of water molecule orientation on the temporal axis, the frequency of random steps will decrease and the diffusion coefficient will become smaller. Geminate recombination of pyranine is extremely sensitive to the diffusion coefficient and is probing at a volume comparable with dw (1). Thus any change of the diffusion coefficient will affect the dynamics of the observed signal.

The dynamics of geminate recombination between H^+ and the excited anion of pyranine has been analyzed by N. Agmon's algorithm. This algorithm calculates the probability density of a proton around an anion by a numerical integration of the Debye Smoluchowski rate equations (1). The analysis is carried out by adjusting molecular parameters, like diffusion coefficient and dissociation-recombination rate constant until a theoretically reconstructed curve is superpositioned over the experimental measurement.

We have adopted Agmon's algorithm by setting a boundary condition operating at $r=dw/2$. At that distance the diffusion space is shifted to annular configuration and the surface reaction with membrane becomes operative. The value of dw is taken from the published data of Parsegian, Rand and their colleagues (2) while the surface reactivity and diffusion coefficient are adjustable parameters.

Figure 1 depicts experimental measurements and the overlaid theoretical reconstructed dynamics. Frame A depicts results measured with DPPC multilamellar vesicles (MLV) where dw was reduced by osmotic pressure from 19.6A (no pressure) to ~ 10A. Frame B records experiments with DPPC-Cholesterol (1:1) MLV. The width of dw in this preparation varies from 29.6A (no pressure) to 12A.

The presentation in Figure 1 is, actually, a time scan of proton probability density at the origin of its diffusion path. In the same way we can calculate the radial distribution of proton at given time points during the evolution of the reaction. Figure 2 depicts this function as protons dissociate in the aqueous lamella between DPPC-Cholesterol membranes. Frame A depicts the diffusion in unpressed preparation. The point where $r=dw/2$ is clearly marked in the tracing. It is the distance where annular diffusion proceeds. As the volume increment in annular space (dv/dr) is unequal to that of spheric one, the steepness of the probability density changes. In highly pressed preparation the propagation of proton is severely restricted. The protons are effectively delayed at the immediate vicinity of the anion (Figure 2B).

Under high compression the tendency of protons to react with the phospho anion is enhanced (see Figure 3, square symbols). As a result the rate of proton loss from the aqueous phase is also a function of the width of the water layer. As seen in the inserts to figure 2, under no pressure the probability density of free proton (summed over all space) decreases to 70% during the first

1.8ns. Under high osmotic pressure within the same time frame the free proton probability is reduced to 10%.

Figure 3 summarizes the effect of compression on the dynamics of proton transfer. At low osmotic pressure the reduced width of the water layer is the only reason for the change in the observed dynamics. It is a regime where the geometry of the reaction space is the single factor modulation of the velocity of the reaction. At higher pressures the water molecules remaining between the membranes are those strongly immobilized by the hydration forces. Accordingly their capacity to exchange proton among them is reduced and their tendency to transfer it to the phospho anion is increased.

It is of interest to point out that the diffusion coefficient of H^+ , as measured in water layers between DPPC or DPPC+Cholesterol, is a function of the activity of water in the layer (Figure 4), not of the width. This observation calls for further elaboration and theoretical evaluation.

References

1. Pines, E., Huppert, D. and Agmon, N. J. Chem. Phys. 88: 5620-5630, 1988.
2. Lis, L.J., McAlister, M., Kuller, N., Rand, R.P. and Parsegian, A.V. Biophys. J. 37: 657-7666, 1982.

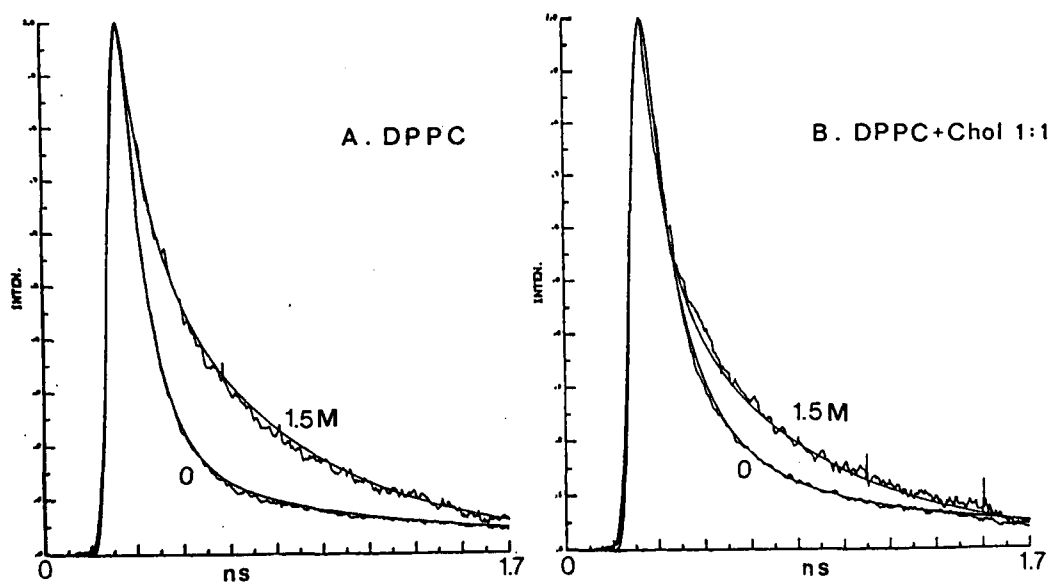


Figure 1. Time resolved fluorescence measurements of pyranine in the thin water layer of multilamellar vesicles made of DPPC (Frame A) or DPPC+Cholesterol (1:1) (Frame B). The concentration of sucrose added as osmosante is indicated in the figure. Each line consists of experimental recording and superpositioned theoretically reconstructed dynamics.

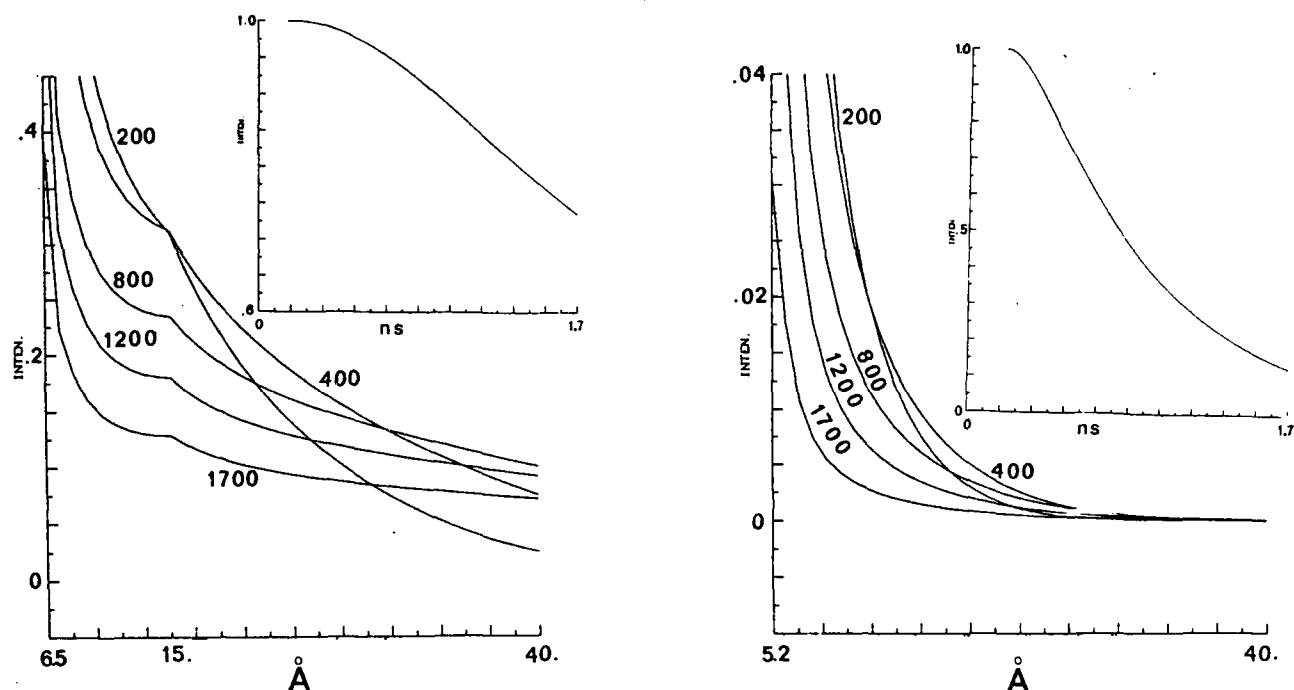


Figure 2. The radial distribution of proton after dissociation in thin water layer between DPPC+Cholesterol membranes. Frame A, unpressed membranes, corresponds with curve 1 in Figure 1B. Frame B is the same as frame A but under osmotic pressure $\log \Pi = 7.8$ dyn/cm² applied by 1.5M sucrose (corresponding with line 2 in Figure 1B). The curves represent radial distribution of protons after 200 ps, 400 ps, 800 ps, 1200 ps and 1700 ps. Insert: The summed probability-density of free proton as function of time. Note the different scale in the two inserts.

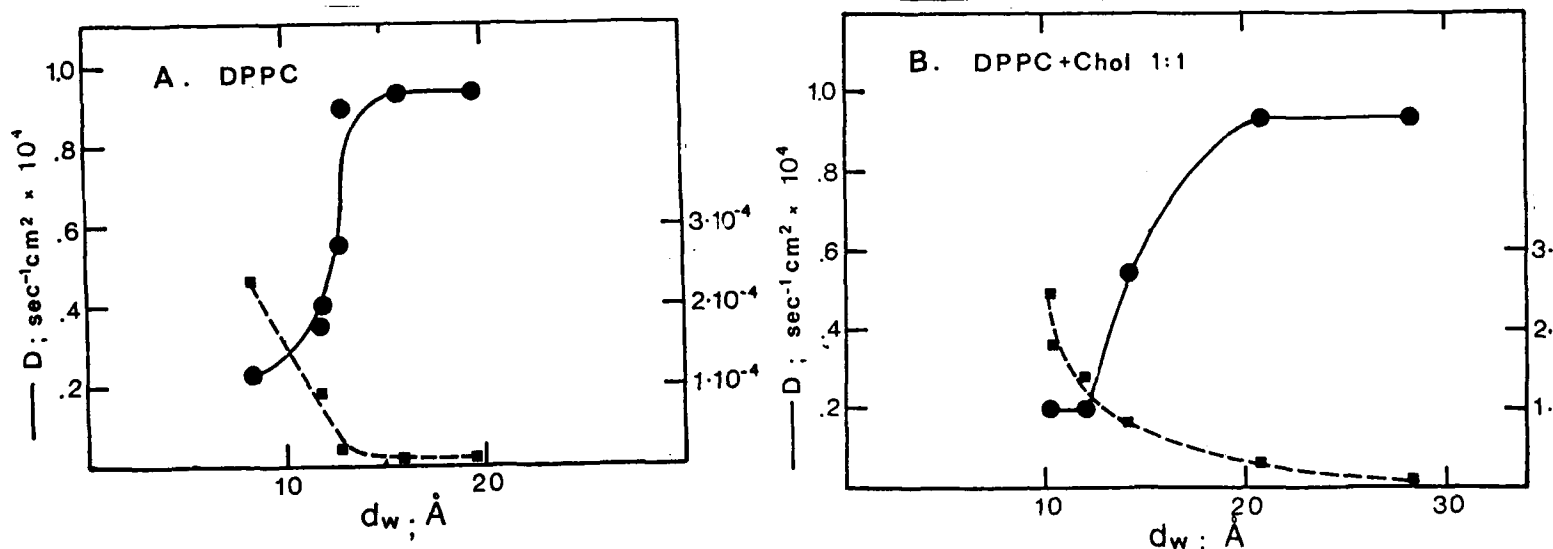


Figure 3. The variation of diffusion coefficient (●) and proton-membrane reactivity (■) as function of the width of the water layer. A) DPPC multilamellar vesicles; B) DPPC+Cholesterol multilamellar vesicles.

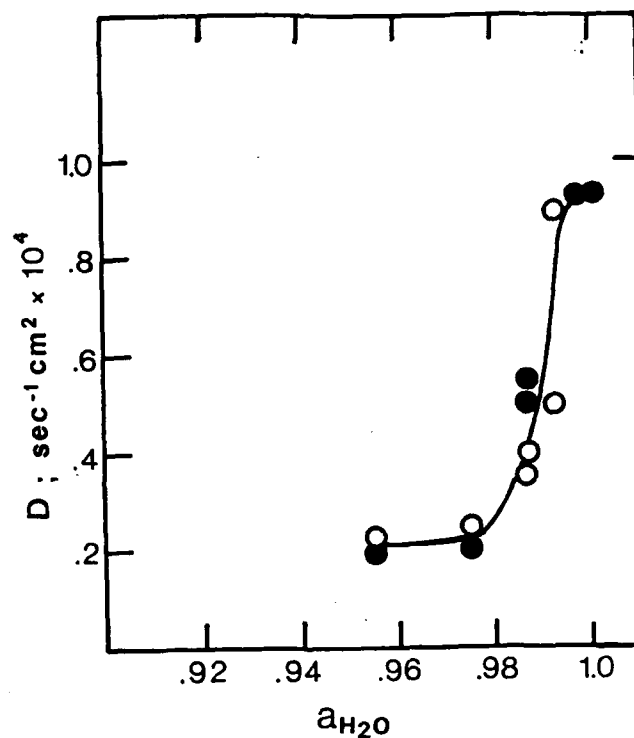


Figure 4. The variation of the diffusion coefficient of proton on the activity of water on the water layer width. (O) measurements with DPPC vesicles; (●) measurement with DPPC+Cholesterol (1:1) vesicles.

DISTRIBUTION LIST

Dr. Igor Vodyanoy, Code 1141SB
Scientific Officer, Biophysics
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000.

Administrator
Defense Technical Information Center
Building 5, Cameron Station
Alexandria, VA 22314.

Mr. Fred E. Walters
Administrative Contracting Officer
ONR Resident Representative
33 Third Avenue, lower level
New York, NY 10003-9998.

Dr. Robert J. Nowak, Code 1113ES
Scientific Officer, Electrochemical
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000.

Program Manager
Biological/Human Factors Division
Code 125
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000.

Program Manager, Defense Technical Support
Technology Directorate
Office of Naval Technology, Code 223
800 N. Quincy Street
Arlington, VA 22217-5000

Commander
Chemical and Biological Sciences Division
Research Army Research Office, P.O. Box 1221
Research Triangle Park, NC 27709.

Head
Biomolecular Engineering Branch
Code 6190
Naval Research Laboratory
Washington, DC 20375.

Directorate of Life Sciences
Air Force Office of Scientific Research
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